Advances in ancient DNA research can help radiological interpretations of archaeological diseases

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Evolutionary Medicine explores among others the human vulnerability towards pathogens. To increase the diagnostic level of evidence, alongside radiological investigations, more often molecular techniques (such as aDNA analyses) are being applied.

Ancient DNA (aDNA) is DNA recovered from archaeological and historical specimens. This DNA is highly degraded and fragmented by biological and environmental factors and is therefore difficult to analyse. However, sequences retrieved from ancient material are highly useful in many ways, including for medical purposes.

The first aDNA experiment was conducted in 1984 to determine the biological classification of the extinct quagga, revealed to be a close relative of zebra (Higuchi *et al*., 1984). In the 1980's and 90's there were multiple publications claiming to have extracted aDNA from a variety of samples. However, some of these claimed sequences were later determined to be the result of modern contamination. This led to the introduction of vigorous anti-contamination procedures in the field of aDNA research. At this time a greater understanding of the need for hypothesis lead research allowed for a great diversity of targets. Ancient DNA has been used for extensively, for example, to clarify the domestication events of animals and plants, to understand the movements of humans, and to examine the history of modern and ancient disease.

The first pathogens targeted by ancient DNA researchers were the *Mycobacterium tuberculosis* complex (MTBC) organisms, and this DNA has proven to be one of the most robust of all the pathogens studied in archaeological remains. Tuberculosis is a chronic infection caused by any member of the MTBC, which are very similar genetically. Tuberculosis can cause numerous bone changes, most significant in Pott's disease, where the vertebrae are pitted and gradually undergo extensive osteoblastic activity resulting in spinal collapse and cause a hunched posture. Whilst tuberculosis can be inferred from visual (including radiological) inspection of archaeological remains, bone changes only occur in a minority of cases. In addition, the actual causative bacteria can not be known without microbiological testing.

Many groups have amplified and sequenced archaeological MTBC DNA, and increasingly try to identify the member of the complex; strain identification is easier than type identification as less genetic information is required. Interestingly, so far, only one archaeological tuberculosis case has been shown to be caused by *Mycobacterium bovis* strain despite the close bovine-human habitation of the past.

Modern geneticists are increasingly studying the phylogeography of MTBC, i.e. the co-relation between strain and it's location. This involves examining multiple mutations from one sample. Traditional polymerase chain reaction (PCR) methods work by targeting specific DNA using primers (short pieces of nucleotides that attach to the complimentary sequence) and copying the DNA in-between, the DNA is then sequenced in a separate experiment. Multiple primers can be used in one reaction but generally this only amplifies a few targets resulting in hundreds or a few thousand bases of DNA sequences. Therefore, it is highly unlikely that enough informative information could be gathered from one degraded archaeological sample to properly place an ancient bacteria into a phylogeographic type.

Recently introduced into the research field of aDNA studies, Next Generation Sequencing (NGS) works by taking the DNA and adding short sequences to either end that are complimentary to universal primers and then amplifying and sequencing each DNA strand individually on a plate so that thousands of strands can be analysed simultaneously (see Mardis, 2008 for an overview). Therefore, the development of NGS systems allow for thousands of targets and millions of bases to be sequenced at one time.

Using one of these NGS systems (SOLiD), MTBC DNA from a 19th century British individual has been typed and identified as a similar, but different type to that isolated in North America in 1905 known as H37Rv (Bouwman *et al*. 2012). One hundred other individuals from the last 2,000 yrs across Europe are stillbeing analysed, the bone changes and strain identification are being compared to see if there is any correlation between the strain and the pathology.

Next generation sequencing has opened up a new field of research to the evolutionary medicine investigator and will continue - alongside radiological diagnostics - to expand our understanding of our past and present health.

Literature:

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